Colony PCR

Materials and equipment

- Thermocycler
- Microtubes (0.2 ml)
- Micropipette tips
- Micropipettes

Reagents

- Template DNA (colony of interest)
- Standard reaction buffer 5X
- dNTPs
- Forward primer (VF2)
- Reverse primer (VR)
- DNA polymerase
- Nuclease-free water

Methodology

1. Prepare DNA template.
2. Take a piece of colony with a micropipette tip.
3. Resuspend in 30µL of nuclease-free water.
4. Thaw the template DNA, Standard reaction buffer 5X, dNTPs and primers (FP, RP) on ice.
5. Add in a 0.2 ml tube (for a 25µL reaction):
6. Up to 25 µL nuclease-free water
7. 5µL Standard Reaction buffer
8. 0.5 µL of 10 mM dNTPs
9. 0.5 µL of 10 µM Forward Primer
10. 0.5 µL of 10 µM Reverse Primer
11. 1µL of Template DNA
12. 0.125 µL DNA Polymerase
13. Mix gently each tube (by pipetting up and down several times).
14. Place at thermoblock in a program, based on the polymerase and primers specifications:
15. Denaturation step: 94˚C, 10 minutes
16. Second Denaturation: 94˚C, 30 seconds
17. Annealing: 56˚C, for 30 seconds
18. Extension: 68˚C, 1 minute
19. Final extension:68˚C, 5 minutes
20. Hold: 4˚C, infinite time
21. Repeat for 30-35 cycles from b-d.
22. Store PCR products at -20˚C.